REMARKS

Claims 6, 10-11, 13, 19-30, 32-86, 88-89, 91-93, and 97-102 have been cancelled without prejudice, and claims 7, 8, 17, 31, 90 and 103 have been amended to define Applicants' invention with greater particularity. The amendments do not add new matter, nor raise new issues, and are fully supported by the Specification and original claims. Accordingly, claims 1-5, 7-9, 12, 14-18, 31, 87, 90, 94-96 and 103 are currently pending in this application.

The Rejection under 35 U.S.C. § 112, Second Paragraph

Claims 7, 8, 12 and 90 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Applicants respectfully traverse this rejection.

Claims 7 and 8 have been amended, and the term "by" has been replaced with the term "while". Hence, claims 7 and 8 have been amended to improve their form and make clear the metes and bounds of the claimed subject matter.

Claim 90 has been amended to correct dependency. Claim 90 is now dependent on pending claim 87, and not cancelled claim 89.

In view of the amendments, Applicants submit that the pending claims meet all requirements under 35 U.S.C. § 112, second paragraph.

The Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 1-9, 12, 14-18, 31, 87, 90, 94-97 and 103 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly not being described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it most nearly connected, to make and/or use the invention. Applicants respectfully traverse this rejection.

According to the Office Action, the test for enablement is whether one skilled in the art can make use of the claimed invention coupled with information known in the art without undue experimentation (page 3). The Office has applied the standard of undue experimentation based on the factors in In re Wands, including: 1) scope/breadth of claims; 2) nature of the

invention; 3) state of the art and predictability; 4) amount of guidance provided; 5) number of working examples; and 6) amount of experimentation required. In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988). The factors will be addressed in the order they were presented in the Office Action.

Scope and breadth of claims. The Office Action states that the claims are broad because they are directed to administration of *any* ABM or ABM conditioned media to heart and limb tissue. Applicants respectfully traverse that the claims are not too broad.

The claims are not too broad because the extraction and harvest of ABM was known in the art at the time of filing. For example, the process of removing healthy bone marrow from a subject for re-injection back into the subject is standard practice for the treatment of various diseases (e.g. cancer patients who receive high-dose radiation treatment). It is customary practice to extract the bone marrow used in autologous bone marrow transplants from a subject's hip bone (i.e. iliac crest). Harvesting of ABM cells from the iliac crest is also described in Fuchs et al. (2003), which reference was enclosed in the response to the last Office Action mailed March 24, 2004. Because the isolation of ABM is not unique to the claimed invention, a particular ABM population need not be delineated. Further, after amendment of the claims, methods of administering ABM or ABM in conditioned media into "limb tissue" have been deleted. Hence, only methods of administering ABM or ABM in conditioned media into "heart tissue" remains.

The Office Action also states that claims drawn to stimulating ABMs *ex vivo* with *any* stimulant (i.e. claim 7, 8 and 17) is too broad. Claims 7, 8 and 17 have been amended, and after the amendments, the claims recite that the harvested ABM cells are growing in conditioned medium comprising granulocyte-monocyte colony stimulating factor (GM-CSF), endothelial PAS domain 1 (EPAS1) and hypoxia inducible factor (HIF-1). Support for the amendments to claims 7, 8 and 17 are found throughout the specification, particularly in Examples 1, 5 and 6.

Accordingly, the scope and breadth of the claims is not too broad and clearly defines the metes and bounds of the claimed invention.

Nature of the invention. After amendment of the claims, the invention encompasses methods of administering ABM alone or ABM in conditioned medium to heart tissue. The invention also encompasses ABM compositions which promote and/or stimulate angiogenesis or blood vessel formation.

State of the art and predictability. The Office Action states that administering ABM alone or ABM in conditioned media is unpredictable, and that the "data is too preliminary to draw definitive conclusions regarding safety and efficacy (e.g. predictability of using the claimed invention)." See page 4 of the Office Action. Applicants respectfully traverse that the claimed invention is unpredictable.

First, as a matter of policy, if patent applicants were forced to wait until the instant the FDA acknowledges the efficacy and safety of novel scientific research, the patent office would be rewarding those who have made minor contributions but filed late, to the detriment of true pioneers in the field who filed early; and who either no longer have pending applications, or have lost a significant portion of their patent term. Such a policy irrevocably harms those who have laid down the crucial framework in the art by making the key discoveries in first place. In effect those at the forefront of research would be forfeiting their intellectual property rights and such policy is contrary to the mission of the Office to promote the "progress of science and the useful arts." Under this policy, inventors have no incentive for early disclosure and it encourages pioneer inventors to conceal their inventions. Further, the Office admits that "injecting culture media into patients to enhance angiogenesis is novel (page 6)." Therefore, Applicants should not have to wait until the method becomes "routine" in order to file for patent rights. The enablement requirement is established as long as the invention discloses and gives sufficient guidance for one of ordinary skill in the art to perform the claimed invention. Applicants argue that the instant claimed invention meets this standard.

Second, there are many reported successful ABM transplants in humans. Recently, a study has shown that intramyocardial injection of ABM cells is a viable treatment for subjects with ischemic hearts. See Exhibit A: "Study suggests intramyocardial injection of cells from bone marrow might be an alternative for heart transplantation," press release, cited in

http://www.news-medical.net/print_article.asp?id=4396; and Exhibit B: Dohmann et al., "Two years follow-up evaluation of functional capacity in end stage ischemic failure patients after autologous bone marrow mononuclear cells transplants," Abstract 1690, European Society of Cardiology Congress 2004, cited in http://cic.escardio.org/AbstractDetails.aspx?id=13781. The original report describing the potential therapeutic method is also attached in Exhibit C: Perin et al., "Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure," Circulation, 107(18): 2294-2302 (2003). Perin et al. was previously cited by the Office in the Office Action mailed March 24, 2004.

Combined, the reports in Exhibits A, B and C show that intramyocardial ABM transplant injection to ameliorate the effects of severe, chronic ischemic heart failure is more than just a potential therapy, rather AMB transplantation to rescue subjects with ischemic heart failure is a viable alternative. Of the ten patients who reached the 2-year follow-up time, seven of these patients showed significant improvement in exercise capacity (see Exhibit B). Moreover, Dohmann et al. and of Perin et al. use catheter injection methodologies similar to that of the claimed invention. For example, to perform the myocardial injections, Dohmann et al. and Perin et al., both use the Myostar catheter by NOGA. This is the same catheter injection procedure utilized by the claimed invention (see page 25, lines 23-25 of specification). In another example, to determine the efficacy of the ABM transplant, Dohmann et al. and Perin et al., describe improvements in exercise capacity of the subjects. Similarly, Applicants' specification describes a ~62% -73% improvement in blood flow in the experimental group compared to that of the control. Increase in blood flow is a biological effect of increased exercise capacity. Thus, the methods of the claimed invention are in effect shown to be efficacious by the teachings of Dohmann et al. and Perin et al.

Thus, Applicants traverse the Office Action's claim that the data is too preliminary or that the art is unpredictable. The discussion above demonstrates that the method of Dohmann et al. and Perin et al., which are partly analogous to the methods of the claimed invention, are safe and efficacious.

Accordingly, the methods of the claims are not unpredictable.

Amount of guidance provided. The Office Action states that there is lack of guidance as to administration of conditioned ABM *in vivo* and administration of ABM to ischemic limbs. Claims 6 (i.e. ABM injection into limb tissue) has been cancelled. Claims 31 and 103 have been amended and "limb" has been deleted. Hence, Applicants respectfully traverse that there is insufficient guidance in the specification to allow one skilled in the art to perform the claimed invention in heart tissue.

Sufficient guidance is provided in Example 1 (method of making conditioned media and detecting endothelial cell proliferation by assaying for levels of MCP-1 and VEGF); Example 3 (method of stimulating vascular tube formation *in vitro* by administration of conditioned medium); Example 4 (*in vivo* method of transendocardial catheter delivery of ABM in ischemic pig hearts and ~50% increase in wall thickening of the ischemic heart wall and 62-73% increase in blood flow of effected ischemic heart tissue therein); Example 5 (method of pre-administration of GM-CSF to ischemic pig hearts to stimulate blood flow and contractility); and Example 6 (method of treating a human subject using standard methodologies in the art and that provided for from studies using the pig animal model). These examples are sufficient to guide one of ordinary skill in the art to perform the claimed invention.

Further, in In re Brana, *in vivo* human data was not necessary because there was reasonable basis for assuming the efficacy of methods in the mouse model was sufficient to meet enablement requirements. In re Brana, 51 F.3d 1560, 34 USPQ 2d 1436 (Fed. Cir. 1995). Similarly, in the claimed invention, there is a reasonable basis for assuming that the method of administering AMB alone, and that of administering ABM conditioned in medium, *in vitro* and in the pig model are operable. Hence, *in vivo* human data is not necessary in order to meet the enablement requirement. Therefore, as in In re Brana, one of ordinary skill in the art can assume that the methods and disclosures of the claimed invention would be operable for their intended purpose when used in humans.

Accordingly, the level of guidance is sufficient for one of ordinary skill in the art.

Number of working examples. The Office Action states that there are no relevant working examples with regard to administration of ABM to limb tissue or administration of

ABM in conditioned medium to limb or heart tissue. Claim 6 (i.e. ABM injection into limb tissue) has been cancelled and claims 31 and 103 have been amended to delete recitation of the term "limb tissue." With regards to lack of a working example for method(s) of administration of ABM in conditioned medium to heart tissue, the ruling in In re Brana supports the present claimed invention in that sufficient *in vitro* and animal [pig] model methods are provided in the specification and *in vivo* human example is not necessary to meet the enablement requirement. Please refer to the discussion above.

Further, the training materials for examining patent applications states that "[t]he presence of only one working example should never be the sole reason for making a scope rejection, even though it is a factor to be considered along with all the other factors. To make a valid rejection, one must evaluate all the facts in evidence and state why one would not expect to be able to extrapolate that one example across the entire scope of the claims." Training Materials for Examining Patent Applications with Respect to 35 USC § 112, first Paragraph-Enablement Chemical/Biotechnical Applications, reprinted in 2 Iver P. Cooper, *Biotechnology Law*, App. H-156, App. H-177 (2000). Therefore, the Office must state exactly why the existing examples cannot be expected to be able to be extrapolated across the entire scope of the claims. That is, the Office has not met its burden of proof.

Accordingly, there is a sufficient number of working examples.

Amount of experimentation required. The Office Action states that the level of skill in the art required to perform the claimed invention is high and that in order to practice the claimed invention, one skilled in the [high] art would have to conduct undue experimentation. Applicants assert that although the skill level is high, there are sufficient examples and guidance in the specification to practice the claimed invention, as discussed above. Therefore, Applicants respectfully traverse that the amount of experimentation is not undue. Further, that in the area of biochemistry and biotechnology there is more unpredictability than in the mechanical or electrical arts, and that the Office cannot use the same standard as in the mechanical or electrical arts to state why enablement is not met. For example, it is now accepted that the Kohler-Milstein process for production of monoclonal antibodies is not 100%

efficient and that a certain amount of experimentation on the part of one skilled in the art is expected for the purpose of producing such antibodies; and that such experimentation is not "undue experimentation." See also, Johns Hopkins University v. Cell Pro. Inc., 47 USPQ 2d 1705 (Fed. Cir. 1998).

Accordingly, although there may be a certain amount of experimentation to perfect the claimed invention, such experimentation is not undue.

The Rejection Under 35 U.S.C. § 102(b)

Claims 87, 94 and 95 are rejected under 35 USC § 102(b) as being anticipated by U.S. Patent No. 5,610,056 to Nakahata (Nakahata). Applicants respectfully traverse this rejection.

Nakahata teaches co-administration or sequential treatment using IL-3 variants with various colony stimulating factors (i.e. cytokines, lymphokines, interleukins, hematopoietic growth factors), which may have the potential for therapeutic use in restoring hematopoietic cells to normal amounts. See Abstract.

Nakahata does not teach ABM compositions conditioned with angiogenic growth factors. Nakahata does not teach that ABM in conditioned medium promotes and stimulates angiogenesis or blood vessel formation. That is the subject matter of claims 87, 94 and 95.

The teachings of Nakahata are distinguished from the claimed invention in several aspects. First, although Nakahata states a "[s]tem cell factor in combination with interleukin-6 and soluble interleukin-6 receptor supports *proliferation, differentiation and terminal maturation* of erythroid cells from normal human hematopoietic stem cells (emphasis added; see Abstract)," Nakahata is not enabling for compositions that promote or stimulate angiogenesis. Second, in In re Donahue, the court states that the prior art in order to anticipate must describe the claimed invention to have placed the public in possession of it. In re Donahue 766 F.2d 531, 533 (Fed. Cir. 1985). Nakahata does not describe the claimed invention to place the public in possession of it, because in fact it does not describe a composition promoting angiogenesis or endothelial cell proliferation, migration or blood vessel formation. Methods of Nakahata are aimed to promote production of erythroid cells from a heterogeneous population of hematopoietic cells. Lastly, it appears that the Office Action is

rejecting claims 87, 94 and 95 based on inherency. If this is the case, inherency anticipation must still meet the requirements of other "anticipation" rejections. Thus, having isolated stem cells alone in conjunction with various interleukins (Nakahata) does not enable a method of using ABM cells to promote angiogenesis or blood vessel formation as in the claimed invention. The skilled artisan would not have recognized that the teachings of Nakahata would promote angiogenesis. Therefore, the compositions of Nakahata do not anticipate the compositions of the claimed invention, because Nakahata does not anticipate that their compositions promote angiogenesis nor does Nakahata use factors which promote angiogenesis. Also, the intended purpose of Nakahata is to support proliferation, differentiation, and terminal maturation of erythroid cells.

Accordingly, claims 87, 94 and 95 are in condition for allowance, and such action is hereby respectfully requested.

The Rejection Under 35 U.S.C. § 102(e)

Claims 87, 94 and 95 are rejected under 35 USC § 102(e) as being anticipated by U.S. Patent No. 5,997,860 to Bauer (Bauer). Applicants respectfully traverse this rejection.

Bauer teaches human interleukin-3 (hIL-3) variant or mutant proteins (muteins) functionally co-administered with other colony stimulating factors (CSF), cytokines, lymphokines, interleukins, hematopoietic growth factors or IL-3 variants. See Abstract. Bauer also teaches that *in vitro* uses include the ability to stimulate bone marrow and blood cell activation and growth before infusion into patients.

Bauer does not teach that ABM in conditioned medium promotes and stimulates angiogenesis or blood vessel formation. That is the subject matter of claims 87, 94 and 95.

Similar to Nakahata, the compositions taught by Bauer are for triggering/stimulating immature bone marrow cell progenitors and not for promoting angiogenesis. In fact, neither Nakahata nor Bauer teach that their compositions are involved in promoting/stimulating angiogenesis or blood vessel formation. That is, neither prior art reference is enabling for compositions which promote/stimulate angiogenesis.

Accordingly, claims 87, 94 and 95 are in condition for allowance, and such action is hereby respectfully requested.

Conclusion

In view of the above amendments and remarks, Applicants submit that all rejections and have been overcome. Accordingly, reconsideration and favorable action on all pending claims are respectfully requested. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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• Home Page

Study suggests intramyocardial injection of cells from bone marrow might be an alternative for heart transplantation

Posted By: News-Medical in Medical Study News
Published: Monday, 30-Aug-2004

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Cardiovascular disease remains the leading cause of morbidity and mortality around the world and patients with end-stage ischemic heart failure carry the highest morbid-mortality rate.

Although heart transplant improves the outcomes of selected patients, the donor heart availability has limited its widespread utilization. Autologous bone marrow mononuclear cells transplantation through intramyocardial injections has been used in very initial clinical trials with promising results for treating these patients.

Our study was carried out at Pró-Cardíaco Hospital with the financial support of the Filantropic Foundation for Teaching and Researching of Pró-Cardíaco Hospital (PROCEP), in Rio de Janeiro, Brazil. The catheter-based injection system (NOGA system, Cordis Johnson&Johnson) was handled by Dr Emerson Perin from Texas Heart Institute, Houston, who was the co-principal investigator in this study.

Twenty-one patients were enrolled in this clinical trial started in December 2001, which was conducted at Pró-Cardíaco Hospital, in Rio de Janeiro, Brazil, in a partnership with Texas Heart Institute, Houston, and the Federal University of Rio de Janeiro.

It is important to emphasize that this trial was preceded by experimental models developed in partnership with the Federal University of Rio de Janeiro, granted by the Brazilian Ministry of Science and Technology. The clinical trial was approved by the Hospital Pró-cardíaco ethics committee and the Brazilian Research Ethics Council.

All patients had severe ischemic heart failure not amenable to any revascularization procedure. Fourteen patients were submitted to injections of autologous bone marrow mononuclear cells into myocardial safely, as our previously published data. The seven other patients served as a comparison or control group, and did not receive injections up to one year follow up. Our main objective was to create new small vessels in myocardial areas that were not receiving enough blood supply and because of that were contracting badly.

According to our published results, the treated patients had a significant 71% reduction in the amount of cardiac muscle with impaired blood supply and an improvement in the mechanical function, compared to the control group.

We came to this ESC Congress to report results from a subgroup of 5 treated patients that were already listed for heart transplantation and we could observe the same benefit that was seen in the entire group, including functional

capacity and quality of life improvement.

We had already reported a six month follow up of such five patients in the 2003 ESC Congress, but there were concerns about the fading of benefits initially observed.

Nevertheless it is intriguing that the exercise capacity not only improved significantly but also reached such threshold that 4 from 5 patients were no longer eligible for cardiac transplantation up to one year follow up.

We have an ongoing post-mortem anatomopathological analysis and immunohistochemical findings from one patient who died at 11 months follow up due to stroke (not from the heart transplantation list) that will give us further understanding of cell mechanisms and behavior after transplantation in humans.

My words from 2003 regarding the social relevance of this finding still seems to be very promising since there is not a single heart transplant program anywhere in the world which is able to treat all the patients who need it.

We all are eagerly awaiting larger clinical trials that could confirm our results, which could lead to a revolutionary treatment for all these end-stage patients on the heart transplantation waiting list.

This release accompanies both a presentation and an ESC press conference given at the ESC Congress 2004.

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ABSTRACT DETAILS

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Last update on : 31 Aug 2004

Abstract: P1690

Two years follow-up evaluation of functional capacity in end stage ischaemic heart failure patients after autologous bone marrow mononuclear cells transplantation

Authors:
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Topic(s):
Congestive heart failure, other
Prognosis (Syncope)

Background: Bone marrow autologous mononuclear cell transplantation (BMMNCT) has been proposed as a potential therapeutic method. In this study we report the 2 year follow up results of the first 9 patients reported in Circulation 2003;107:2294.

Methods: Fourteen pts (60±10yrs, 12 male) with refractory symptoms (CCS/NYHA III-IV), LV dysfunction (EF 19±10%) were included in the published study. Bone marrow (50 ml) was aspirated and BMMNCs were isolated by Ficoll Hypaque gradient. Transendocardial injections (15±2 sites, 0.2 cc) were peformed using the Myostar catheter (NOGA, Cordis®). The first ten patients reached the 2 years follow up time. Patients #3 and #9 have died during this time course. The first one was related in the original article and the second died from acute cerebral stroke 11 months after treatment. Patient #5 developed peripheral artery disease and was not able to perform the treadmill test. We are reporting the treadmill test results of the 7 remaining patients, 2 years after the injections.

Results: Comparing to baseline the results from this 7 patients (6 male, 55.9±8.7years old) showed a significant improvement in exercise capacity (from 18.8±8.8 ml/kg/min to 26.17±11.1 ml/kg/min, p=0,04). When comparing these results with those of 6 and 12 months follow up, there was no significant improvement in exercise capacity. There was a non significant concomitant improvement in NYHA functional class. These results are summarized in Table I (p related from baseline to 24 months)

Conclusion: These preliminary data suggest that the improvements observed at the first 6 months was maintained up to 24 months. During the meeting, data of all 14 patients including mechanical data will be available. This is the longer follow up ever presented in patients with ischemic heart failure submitted to BMMNCT.

Table I

	Baseline	6 months	12 months	24 months	p

VO2	18.8±8.8	24.9±9.3	25.9±11.2	26.1±11.1	0,04
NYHA	2.14±0.83	1.5±0.93	1.6±1.03	1.57±0.97	0.11

Exercise and clinical evaluations during time

Transendocardial, Autologous Bone Marrow Cell Transplantation for Severe, Chronic Ischemic Heart Failure

Emerson C. Perin, MD, PhD*; Hans F.R. Dohmann, MD*; Radovan Borojevic, PhD; Suzana A. Silva, MD; Andre L.S. Sousa, MD; Claudio T. Mesquita, MD, PhD; Maria I.D. Rossi, PhD; Antonio C. Carvalho, MD, PhD; Helio S. Dutra, PhD; Hans J.F. Dohmann, MD, PhD; Guilherme V. Silva, MD; Luciano Belém, MD; Ricardo Vivacqua, MD; Fernando O.D. Rangel, MD; Roberto Esporcatte, MD; Yong J. Geng, MD, PhD; William K. Vaughn, PhD; Joao A.R. Assad, MD; Evandro T. Mesquita, MD, PhD; James T. Willerson, MD

Background—This study evaluated the hypothesis that transendocardial injections of autologous mononuclear bone marrow cells in patients with end-stage ischemic heart disease could safely promote neovascularization and improve perfusion and myocardial contractility.

Methods and Results—Twenty-one patients were enrolled in this prospective, nonrandomized, open-label study (first 14 patients, treatment; last 7 patients, control). Baseline evaluations included complete clinical and laboratory evaluations, exercise stress (ramp treadmill), 2D Doppler echocardiogram, single-photon emission computed tomography perfusion scan, and 24-hour Holter monitoring. Bone marrow mononuclear cells were harvested, isolated, washed, and resuspended in saline for injection by NOGA catheter (15 injections of 0.2 cc). Electromechanical mapping was used to identify viable myocardium (unipolar voltage $\geq$6.9 mV) for treatment. Treated and control patients underwent 2-month noninvasive follow-up, and treated patients alone underwent a 4-month invasive follow-up according to standard protocols and with the same procedures used as at baseline. Patient population demographics and exercise test variables did not differ significantly between the treatment and control groups; only serum creatinine and brain natriuretic peptide levels varied in laboratory evaluations at follow-up, being relatively higher in control patients. At 2 months, there was a significant reduction in total reversible defect and improvement in global left ventricular function within the treatment group and between the treatment and control groups (P=0.02) on quantitative single-photon emission computed tomography analysis. At 4 months, there was improvement in ejection fraction from a baseline of 20% to 29% (P=0.003) and a reduction in end-systolic volume (P=0.03) in the treated patients. Electromechanical mapping revealed significant mechanical improvement of the injected segments (P<0.0005) at 4 months after treatment.

Conclusions—Thus, the present study demonstrates the relative safety of intramyocardial injections of bone marrow–derived stem cells in humans with severe heart failure and the potential for improving myocardial blood flow with associated enhancement of regional and global left ventricular function. **(*Circulation.* 2003;107:2294-2302.)**

Key Words: cells ■ heart failure ■ ischemia ■ revascularization ■ gene therapy

After myocardial infarction, chronically ischemic (hibernating) myocardium may persist in association with variable degrees of scar tissue. In most circumstances, native angiogenesis is insufficient to prevent the resultant remodeling when significant injury occurs. As a consequence, infarct-related heart failure remains a major cause of morbidity and mortality.

The understanding that vasculogenesis can occur in the adult has led to intense investigation into stem cell therapy. Several recent experimental studies have confirmed the potential of pluripotential cells in differentiating into cardiomyocytes and endothelial cells.[1,2] Further evidence from animal models has confirmed that pluripotential cells from

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*Drs Perin and Dohmann are co-principal investigators.

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bone marrow improve myocardial function and perfusion in the setting of ischemic heart disease.[3,4] In addition, recent publications[5,6] have described beneficial effects of intracoronary infusion of autologous, mononuclear bone marrow in the immediate postinfarction period in humans. A recent report by Tse et al[7] described improvement in myocardial perfusion and segmental contractility (as assessed by cardiac magnetic resonance imaging) in ischemic myocardial segments treated with catheter-based delivery.

The present study addresses primarily the safety of endocardial bone marrow mononuclear cell (BMMNC) injections and secondarily the hypothesis that endocardial injections of autologous BMMNCs (ABMMNCs) in patients with end-stage ischemic heart disease may promote neovascularization and may overcome the failure of the natural myocardial healing process.

Methods

Patient Population

This is a prospective, nonrandomized, open-label study of 21 patients with severe ischemic heart failure and no other option for standard revascularization therapies. Patients were enrolled sequentially, with the first 14 patients assigned to the treatment group and the last 7 patients to the control group. In accordance with the ethics committee's recommendations, an initial group of 4 patients was enrolled as a safety study. After 4 months' follow-up of the initially injected patients (once safety was determined), the remaining study patients were enrolled. All patients were placed on maximally tolerated medical therapy at time of enrollment. The following inclusion criteria were required for patient enrollment: (1) chronic coronary artery disease with reversible perfusion defect detectable by single-photon emission computed tomography (SPECT); (2) left ventricular (LV) ejection fraction (EF) <40%; (3) ineligibility for percutaneous or surgical revascularization, as assessed by coronary arteriography; and (4) signed, informed consent. Ineligibility for surgical or percutaneous revascularization procedures was determined by 2 expert committees: a surgical committee comprising 2 cardiovascular surgeons and a noninvasive cardiologist, and an interventional committee comprising 2 interventional cardiologists and 1 noninvasive cardiologist. Patients were not enrolled in the study if any 1 of the following exclusion criteria was met: (1) difficulty in obtaining vascular access for percutaneous procedures; (2) previous or current history of neoplasia or other comorbidity that could impact the patient's short-term survival; (3) significant ventricular dysrhythmias (sustained ventricular tachycardia); (4) LV aneurysm; (5) unexplained abnormal baseline laboratory abnormalities; (6) bone tissue with abnormal radiological aspect; (7) primary hematologic disease; (8) acute myocardial infarction within 3 months of enrollment in the study; (9) presence of intraventricular thrombus by 2D Doppler echocardiogram; (10) hemodynamic instability at the time of the procedure; (11) atrial fibrillation; or (12) any condition that, in the judgment of the investigator, would place the patient at undue risk.

The ethics committee of Pro-Cardiaco Hospital (Rio de Janeiro) and the Brazilian National Research Ethics Council approved the study protocol.

Baseline Evaluation

Baseline evaluation in the treatment group included a complete clinical evaluation (history and physical), laboratory evaluation (complete blood count, blood chemistry, C-reactive protein [CRP], brain natriuretic peptide [BNP], creatine kinase [CK]-MB and troponin serum levels), exercise stress test with ramp treadmill protocol,[8] 2D Doppler echocardiogram, dipyridamole SPECT perfusion scan, and 24-hour Holter monitoring.

The control group underwent the above-mentioned baseline evaluation except for 24-hour Holter monitoring, CK-MB, and troponin serum levels.

Periprocedural Evaluation

Patients in the treatment group had serum CRP, complete blood count, CK, troponin, and BNP (only 9 patients) levels measured and an ECG performed just before the procedure. Immediately after the procedure, another ECG and 2D Doppler echocardiogram were performed, and 24-hour Holter monitoring was begun. Serum CRP, CK, and troponin levels were also assessed at 24 hours. Patients were monitored in the cardiac intensive care unit for 48 hours after the injection procedure.

Bone Marrow Aspiration and Isolation of Mononuclear Cells

Approximately 4 hours before the cell injection procedure, bone marrow (50 mL) was aspirated under local anesthesia from the posterior iliac crest. BMMNCs were isolated by density gradient on Ficoll-Paque Plus (Amersham Biosciences). Mononuclear cells were exhaustively washed with heparinized saline containing 5% human serum albumin and filtered through 100-μm nylon mesh to remove cell aggregates. The cells were finally resuspended in saline with 5% human serum albumin for injection. A small fraction of the cell suspension was used for cell counting and viability testing with trypan blue exclusion. Cell viability was shown to be >90% (96.2±4.9%), assuring the quality of the cell suspension. Post-hoc characterization of leukocyte differentiation markers by flow cytometry and functional assays was done on another fraction of cells. The clonogenic capacity of hematopoietic progenitors was evaluated by colony-forming assays (granulocyte-macrophage colony-forming unit) as previously described.[9]

A high correlation between granulocyte-macrophage colony-forming units and $CD45^{lo}CD34^{+}$ cells was seen (Spearman $r=0.77$, $P=0.0012$). Fibroblast colony-forming assay was done as previously described[10] to determine the presence of putative progenitor mesenchymal lineages. Bacterial and fungal cultures of the clinically used cell preparations were performed and proved negative.

Antibodies and Staining Procedure for Fluorescence-Activated Cell Sorter Analysis

The following antibodies were either biotinylated or conjugated with fluorescein isothiocyanate (Pharmingen), phycoerythrin (PE), or PerCP: anti-CD45 as a pan-leukocyte marker (clone HI30), anti-CD34 as a hematopoietic progenitor marker (clone HPCA-II), anti-CD3 as a pan–T-cell marker (clone SK7), anti-CD4 as a T-cell subpopulation marker (clone SK3), and anti-CD8 as a T-cell subpopulation marker (clone SK1) from Becton Dickinson; anti-CD14 as a monocyte marker (clone TUK4), anti-CD19 as a pan–B-cell marker (clone SJ25-C1), and anti-CD56 as an NK-cell marker (clone NKI nbl-1), from Caltag Laboratories (Burlingame, Calif); and anti-HLA–DR (MHC-II, clone B8.12.2) from Beckman-Coulter. The biotinylated antibodies were revealed with Streptavidin PECy7 (Caltag Laboratories). Three-color immunofluorescence analysis was used for the identification of leukocyte populations in total nucleated bone marrow cell suspensions. After staining, erythrocytes were lysed with the Becton Dickinson lysis buffer solution according to the manufacturer's instructions, and CD45 antibody was used to assess the percentages of leukocytes in each sample. Data acquisition and analyses were performed on a fluorescence-activated cell sorter Calibur with CellQuest 3.1 software (Becton Dickinson).

Transendocardial Delivery of ABMMNCs

In the cell-injection treatment group, patients were taken to the cardiac catheterization laboratory ≈1 hour before the anticipated arrival of the bone marrow cells from the laboratory. Left heart catheterization with biplane LV angiography was performed. Subsequently, electromechanical mapping (EMM) of the left ventricle was performed as previously described.[11] The general region for treatment was selected by matching the area identified as ischemic

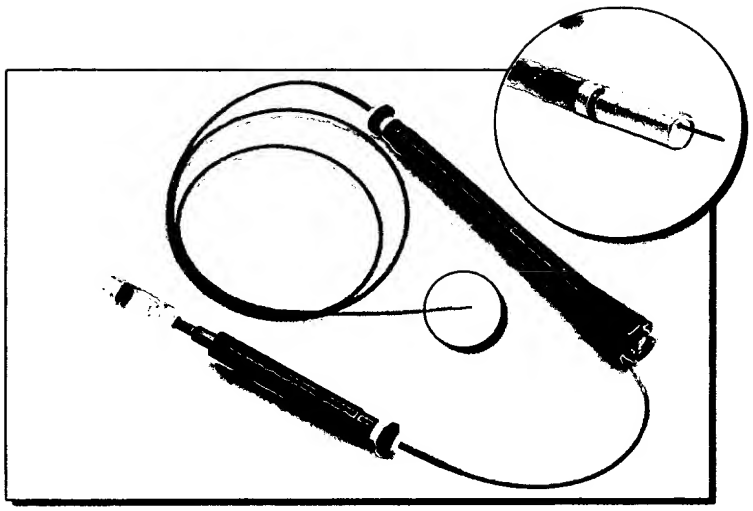

Figure 1. The NOGA Myostar injection catheter, with the needle in the extended position (insert).

by previous SPECT perfusion imaging. The electromechanical map was then used to target the specific treatment area by identifying viable myocardium (unipolar voltage ≥6.9 mV)[12] within that region. Areas associated with decreased mechanical activity (local linear shortening <12%, indicating hibernating myocardium) were preferred.

The NOGA injection catheter (Figure 1) was prepared by adjusting the needle extension at 0° and 90° flex and by placing 0.1 cc of ABMMNCs to fill the needle dead space. The injection catheter tip was placed across the aortic valve and into the target area, and each injection site was carefully evaluated before the cells were injected. Before every injection of cells into the LV wall, the following criteria had to be met: (1) perpendicular position of the catheter to the LV wall; (2) excellent loop stability (<4 mm); (3) underlying voltage >6.9 mV; and (4) presence of a premature ventricular contraction on extension of the needle into the myocardium. Fifteen injections of 0.2 cc (mean of $25.5 \pm 6.3 \times 10^6$ cells/patient) were delivered (Figure 2).

Two-Month Noninvasive Follow-Up Evaluation

All patients, both treated and control, underwent noninvasive follow-up evaluations at 2 months, which consisted of a clinical evaluation, ramp treadmill protocol, 2D Doppler echocardiogram, and dipyridamole SPECT perfusion scan. Patients in the treatment group had repeat 24-hour Holter monitoring. The ramp treadmill protocol was selected because it is better than standard incremental protocols in estimating functional capacity in these severely ill patients.[8]

The predicted $\dot{V}O_2$max was used to tailor the patient workload. Treadmill speed was initially 0.5 mph, and inclination was 0% to 10% with a planned duration of 10 minutes of exercise.[13,14] The echocardiographic data were analyzed by 2 independent, blinded, experienced observers. Images were stored digitally and analyzed offline. If a discrepancy between the readings of >5% was noted, a third blinded observer was called and a consensus achieved. The end-systolic volume (ESV), end-diastolic volume (EDV), and EF were measured according to standard protocols.

Dipyridamole stress and resting SPECT imaging were performed with the same stress procedure at baseline and at follow-up. Studies were read by a blinded, experienced observer. Approximately 740 MBq of technetium-99m sestamibi was injected at rest and after stress, with dipyridamole infusion at a rate of 142 μg/kg of body weight per minute infused for 4 minutes. One hour later, SPECT imaging was initiated, using a 15% window centered over the 140-keV photopeak. Acquisitions were performed with a 1-detector gamma camera (Ecam, Siemens), acquiring 32 projections over 180° (right anterior oblique 45° to left posterior oblique 45°) (low-energy, high-resolution collimation; 64×64 matrixes; and 35 seconds per projection). Short-axis and vertical and horizontal long-axis tomograms of the left ventricle were extracted from the reconstructed transaxial tomograms by performing coordinate transformation with appropriate interpolation. No attenuation or scatter correction was applied. Quantitative SPECT analysis was performed on an ICON workstation computer (Siemens). The analysis was performed with the use of a completely automated software package, with the exception of a quality-control check to verify the maximum count circumferential profiles. The methods for quantitative analysis have been previously described.[15,16] In brief, processing parameters, including the apical and most basal tomographic short-axis slices, the central axis of the LV chamber, and a limiting radius for myocardial count search, were automatically derived. Short-axis tomograms

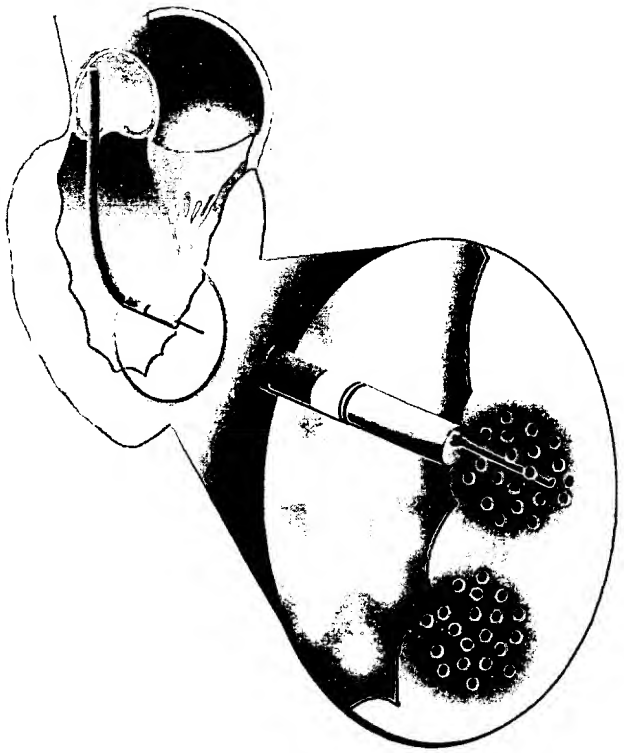

Figure 2. Injection catheter advanced into the left ventricle through the aortic valve. The catheter tip is placed against the endocardial surface (insert) with the needle extended into the myocardium delivering ABMMNCs.

TABLE 1. Demographics of the Treatment and Control Groups

	Treatment (n=14)	Control (n=7)	P
Age	56.9±9.8	64.3±7.2	0.1
Male gender, %	86	90	0.53
Hypertension, %	64	71	0.74
Diabetes, %	29	57	0.35
Hypercholesterolemia, %	79	57	0.35
Smoking, %	7	0	0.47
Previous myocardial infarction, %	100	100	1.0
Previous percutaneous coronary intervention, %	7	43	0.09
Previous coronary artery bypass grafting, %	64	86	0.61
Previous stroke, %	29	0	0.26
Peripheral vascular disease, %	57	71	0.66
Chronic renal failure, %	14	14	1.0
Multivessel disease, %	100	100	1.0

Values are mean±SD or percentage of patients.

were then sampled by using a maximum-count circumferential profile sampling technique with a cylindrical approach for sampling the body of the left ventricle and a spherical approach for sampling the LV apex. Comparisons were made to sex-matched normal limits.[16] Polar map displays and quantitative values were then generated to indicate stress myocardial perfusion defect extent and severity.[16,17]

Four-Month Invasive Follow-Up Evaluation

Patients in the control group did not undergo NOGA mapping or repeat LV angiograms at late follow-up (because of ethics committee recommendations).

Patients in the treatment group had 4-month invasive follow-up evaluations consisting of LV angiograms and EMM. LV angiography was performed through the femoral approach with the use of a 5F pigtail catheter. All angiograms were obtained in 2 planes—a 30° right anterior oblique view and a 60° left anterior oblique view—during a period of stable sinus rhythm. Ventricular volume was not measured during or after a premature beat. A 40-mm sphere was used as calibration device. LV EDV, ESV, and EF were calculated by 2 blinded, experienced observers who used the area-length method.[18]

EMM was performed according to established criteria[11] with a fill threshold of 15 mm. After the acquisition of points, postprocessing analysis was performed with a series of filters (moderate setting) to eliminate inner points, points that do not fit the standard stability criteria (location stability <4 mm, loop stability <6 mm, and cycle length variation <10%), points acquired during ST-segment elevation, and points not related to the left ventricle (eg, those in the atrium). A blinded, expert observer used a 12-segment bull's-eye to compare electromechanical values (unipolar voltage and local linear shortening) of injected segments at baseline and follow-up.

Statistical Analyses

Univariate differences in demographic characteristics (Table 1) between the control and treated groups were assessed with χ^2/Fisher's exact test and *t* tests for discrete and continuous variables, respectively. Multivariable logistic regression was also used to determine the independent relationship between each demographic variable and treatment group. No statistically significant differences between the 2 groups were found. Because each patient in both groups was used as his or her own control, changes between baseline and 8 weeks in the control and treated groups were assessed with paired *t* tests. Logistic regression analysis was utilized to compare medications (Table 2) at baseline, 8 weeks, and 16 weeks within the control and treatment groups and between the control and treatment groups.

TABLE 2. Percentage of Patients Receiving Selected Cardiac Medications at Baseline and 8- and 16-Week Follow-Up

	Baseline	8 Weeks	16 Weeks	P
ACE+ARB				
Control	86	86	86	1.0
Treatment	86	100	93	0.32
P	0.63*			
Nitrates				
Control	86	86	86	0.99
Treatment	93	93	93	0.91
P	0.95*			
β-Blockers				
Control	43	57	57	0.59
Treatment	71	71	64	0.73
P	0.94*			
Diuretics				
Control	71	71	71	1.0
Treatment	86	79	71	0.56
P	0.65*			
Ca channel blockers				
Control	14	14	29	0.49
Treatment	21	29	21	0.89
P	0.62*			

*P for comparison of all 3 time periods between treatment and control groups.

Comparisons of the changes from baseline to 8 weeks in the control and treatment groups were made with repeated-measures ANOVA. The ANOVA model included the control versus treatment and baseline versus 8 weeks as factors and also included the interaction between the 2 factors. A probability value <0.05 was considered statistically significant.

Results

Patient population demographics did not differ significantly between the treatment and control groups (Table 1). There were no significant differences in β-blocker, ACE inhibitor, or nitrate use between the 2 groups (Table 2).

Procedural Data

The total procedural time for mapping and injection was 81±19 minutes. Electromechanical maps comprised an average of 92±16 points. Patients received an average of 15±2 cell injections in a mean of 2±0.7 segments (6 inferior, 14 lateral, 2 anterior, and 5 septal). Each injection of 2 million cells was delivered in a volume of 0.2 cc. The cell population comprised a mean of 2.44±1.33% $CD45^{lo}CD34^{+}$ cells (Table 3).

Safety Data

One patient in the control group died 2 weeks after enrollment in the study and was not included in the analysis. A patient in the treatment group died at 14 weeks, presumably of sudden cardiac death. This patient had onset of severe angina and was found to be in asystole by emergency medical personnel. The patient had persistent improvement in cardiac

TABLE 3. Characteristics of Bone Marrow Mononuclear Cells Injected Into the Myocardium*

Cell Population and Phenotype	Percent of Injected Cells	No. of Cells Injected, ($\times 10^3$)/mm^2
Hematopoietic progenitor cells (CD45^{lo}CD34$^+$)	2.4±1.3*	57.4±61.4*
Early hematopoietic progenitor cells (CD45^{lo}CD34$^+$HLA-DR$^-$)	0.1±0.1	2.1±1.8
CD4$^+$ T cells (CD45$^+$CD3$^+$CD4$^+$)	28.4±10.8	537.0±265.7
CD8$^+$ T cells (CD45$^+$CD3$^+$CD8$^+$)	14.9±5.9	311.0±221.6
B cells (CD45$^+$CD19$^+$)	1.9±1.0	232.5±174.8
Monocytes (CD45$^+$CD14$^+$)	10.0±4.0	202.8±161.0
NK cells (CD45$^+$CD56$^+$)	1.2±0.5	21.2±13.5
Functional assay	**No. Colonies/10^6 BMMNC**	**No. of Cells Injected, ($\times 10^3$)/mm^2**
Fibroblast colony-forming assay	7.8±9.7	0.2±0.2
Granulocyte-macrophage colony-forming unit assay	719.6±385.3	16.4±18.5

Values are average±SD.

*Results for 14 patients in the treatment group, except: CD34$^+$CD45^{lo}HLA-DR$^-$, 13 patients; CD45$^+$CD19$^+$, 13 patients; CD45$^+$CD14$^+$, 11 patients; and CD45$^+$CD56$^+$, 9 patients.

function, as assessed by echocardiography. Baseline EF was 30% by echocardiography and increased to 57% at 2-month follow-up, demonstrating a similar response as the rest of the treatment group with regard to increased contractile function. In both cases, the families refused postmortem exams.

There were no major periprocedural complications. One patient had a transient episode of pulmonary edema that was easily reversed with loop diuretics after the procedure. No sustained arrhythmias were associated with the injection procedures, nor did any significant arrhythmias occur while the patients were hospitalized. There were no sustained ventricular arrhythmias found on 24-hour Holter monitoring at baseline or when repeated after the injection procedure and no significant differences in the number or percentage of premature ventricular contractions. No postprocedural pericardial effusions were seen on 2D Doppler echocardiograms. All patients were discharged on the third hospital day as per protocol.

Two-Month Noninvasive Follow-Up Evaluations

Of all baseline and follow-up laboratory values (Table 4), only serum creatinine and BNP levels varied between the control and treatment groups at follow-up. Follow-up serum creatinine levels were significantly elevated in the control group as compared with the treatment group ($P=0.03$). The levels of CRP at baseline and follow-up were not significantly different between the two groups (Table 4). There was a trend toward increased difference of BNP levels at follow-up between the two groups, with higher levels in the control group ($P=0.06$).

Patients in the treatment group experienced less heart failure and fewer anginal symptoms at the 2-month follow-up when compared with the control group, by both New York Heart Association (NYHA) and Canadian Cardiovascular Society Angina Score (CCSAS) distribution (Table 5). Baseline exercise test variables (METs and $\dot{V}o_2$max) were similar for the 2 groups. There was a significant increase, however, in METs and $\dot{V}o_2$max at follow-up in the treatment group ($P=0.0085$ and 0.01, respectively). There was a trend toward improvement when these variables were compared with the control group ($P=0.08$ for both variables).

Baseline comparison of ESV, EDV, and LVEF between the treatment and control groups revealed significant differ-

TABLE 4. Laboratory Values for the Treatment and the Control Groups

	Treatment (n=14)	Control (n=7)	*P*
White blood cells, nL			
Before treatment	8.3±2.8	8.6±1.5	0.39
After treatment	8.3±2.1	9.2±1.4	0.19
P	0.85	0.14	
Creatinine, mg/dL			
Before treatment	1.17±0.32	1.35±1.02	0.60
After treatment*	1.10±0.26	1.63±0.08	0.030
P	0.23	0.09	
CRP, mg/dL			
Before treatment	1.00±0.70	0.76±0.50	0.43
After treatment	1.03±1.0	0.61±0.57	0.33
P	0.94	0.59	
BNP, pg/mL			
Before treatment	328.1±410.7	404.4±421.6	0.73
After treatment	281.8±286.6	565.1±366.3	0.06
P	0.91	0.19	
CK-MB, ng/mL			
Before treatment	2.67±0.42	NA	NA
24 Hours	3.08±1.43	NA	NA
P	0.35	NA	NA
Troponin, ng/mL			
Before treatment	0.14±0.09	NA	NA
24 Hours	1.13±0.84	NA	NA
P	0.0007	NA	NA

CK-MB indicates myocardial muscle creatine kinase isoenzyme; NA, not applicable.

*After treatment=2 months.

TABLE 5. Comparison of Baseline and 2-Month Follow-Up Values for the Treatment and Control Groups

	Treatment (n=14)	Control (n=7)	P*
NYHA class			
Before treatment	2.21±0.89	2.71±0.75	
After treatment	1.14±0.36	2.71±0.76	0.0001
P	0.0003	1.0	
CCSAS class			
Before treatment	2.64±0.84	2.57±0.97	
After treatment	1.28±0.61	2.14±0.89	
P	0.0001	0.06	0.001
Ramp treadmill METs			
Before treatment	5.09±2.5	5.07±1.96	
After treatment	6.68±2.35	5.16±2.45	0.078
P	0.0085	0.84	
$\dot{V}o_2$max			
Before treatment	17.96±8.78	17.75±6.85	
After treatment	23.38±8.31	18.08±8.58	0.08
P	0.01	0.84	
Echocardiogram			
ESV, cc			
Before treatment	146.78±53.46	89.42±26.23	
After treatment	123.21±47.88	98.85±20.52	0.041
P	0.026	0.36	
EDV, cc			
Before treatment	211.35±76.89	135.71±26.08	
After treatment	189.14±67.54	145±27.62	0.09
P	0.065	0.50	
EF, %			
Before treatment	30±5.56	36±11.73	
After treatment	35.5±7.85	31.85±7.55	0.029
P	0.027	0.31	
SPECT			
Total reversible defect, %			
Before treatment	15.15±14.99	10.71±16.60	
After treatment	4.53±10.61	32.28±37.25	0.022
P	0.016	0.23	
% Rest defect (50%)			
Before treatment	40.77±11.13	35.85±10.09	
After treatment	38.84±8.79	36.42±12.08	0.65
P	0.44	0.77	

*P values reflect comparison of the differences between treatment and control groups over time (see Methods).

ences: The control group had smaller LV volumes ($P<0.001$) and a trend ($P=0.054$) toward higher baseline EF. Cardiac function (measured by EF on echocardiograms) had an absolute increase of 6% over the 2-month follow-up period in the cell-treated group. In contrast, the mean EF decreased, although not significantly, in the control group. In addition, when the 2 groups were compared, the treatment group showed a significant improvement in EF after 2 months ($P=0.03$). Cardiac geometry, as assessed by ESV, also improved. A significant fall in ESV ($P=0.03$) and a trend toward reduction in EDV ($P=0.07$) were noted in the treatment group. Volumes remained unchanged within the control group. When the two groups were compared at follow-up, a significant reduction in ESV was seen in the treated patients ($P=0.04$).

Nuclear perfusion imaging studies were similar at baseline for the amount of total reversible defect and percent of rest defect with 50% activity (scar). Within the control group, there was no significant change in these two variables at follow-up. Within the treatment group, there was no significant change in rest defect, with 50% activity at 2-month follow-up, but there was a significant 73% reduction in total reversible defect ($P=0.022$; from 15.15±14.99% to 4.53±10.61%). A typical example of resolution of inferolateral ischemia (baseline to follow-up) in a cell-treated patient is shown in Figure 3A.

Four-Month Invasive Follow-Up Evaluations

Results from LV angiography at baseline and 4-month follow-up are shown in Table 6. There was a sustained improvement in LVEF from baseline, an increase from 20% to 29% at 4 months (31% relative increase) ($P=0.0003$) in the treated patients. There was also a continued reduction in ESV ($P=0.03$) at 4 months. EDV remained unchanged ($P=0.1$). Control group patients did not have repeat LV angiograms.

On EMM, segmental analysis revealed a significant mechanical improvement of the injected segments ($P<0.0005$) (Table 6). Significant improvement in mechanical function at the injection site is illustrated by EMM in Figure 3B. Unipolar voltage values did not change from baseline to follow-up.

Discussion

The present study describes for the first time ABMMNC transplantation with the use of transendocardial injections in patients with severe LV dysfunction, end-stage ischemic heart disease, and no other option for treatment. The results of our study suggest that injection of ABMMNCs is safe and improves perfusion and myocardial contractility when viable areas of myocardium are targeted.

Wound healing is a multifaceted process that involves complex interactions between inflammatory cells, cytokines, and a number of extracellular matrix proteins, and the development of new capillaries. Because the normal reparative mechanisms seem to be overwhelmed when clinically significant myocardial injury occurs, a logical next step would be to amplify one part of this response artificially by applying stem cells locally in the setting of ischemia or infarction when a large amount of heart muscle has been injured.

In experimental animals, bone marrow–derived cells have been shown to regenerate areas of infarcted myocardium and coronary capillaries,[1] thus limiting functional impairment after myocardial infarction. Transendocardial injection of ABMMNCs has been shown to increase myocardial contractility and perfusion in swine.[4] Various cell lineages have been used to generate evidence that bone marrow stem cells

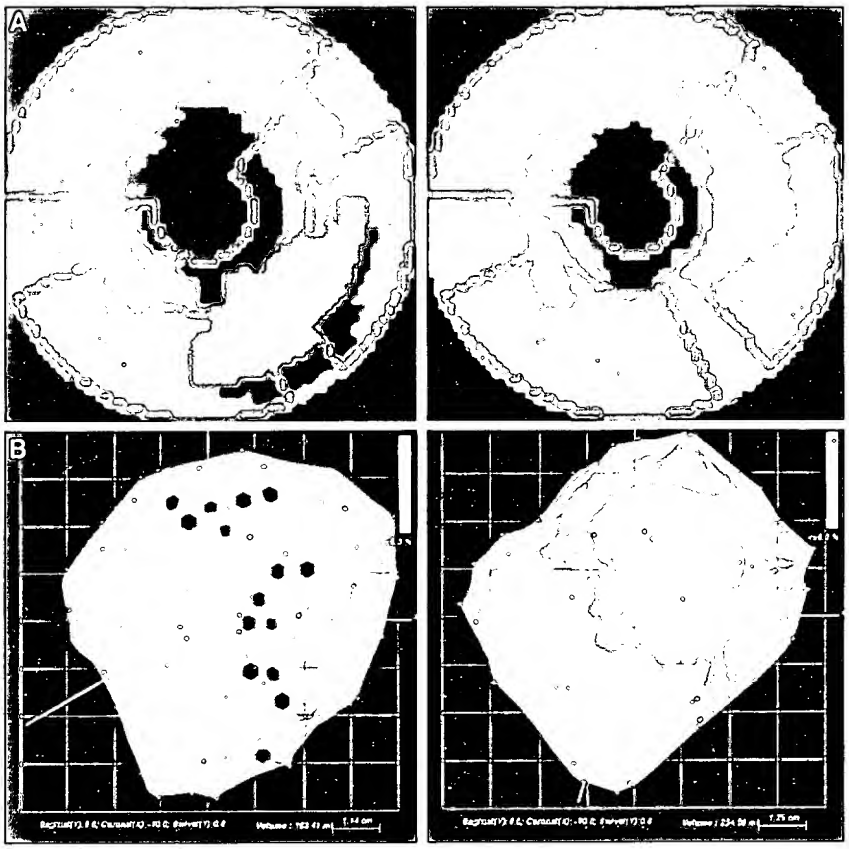

Figure 3. A, SPECT polar map at baseline, showing an area of inferolateral, reversible ischemia in white and nonreversible stress defect in black (left). Follow-up SPECT at 2 months, showing complete resolution of ischemic defect and basilar nonreversible defect with a decrease in nonreversible apical defect (right). B, Electromechanical maps from the same patient viewed from the inferior position. Mechanical map at the time of the injection procedure (left) shows the 15 injection sites in black distributed along the inferior wall. The follow-up mechanical map at 4 months (right) shows marked improvement in contractile function in the injected area.

differentiate into cardiomyocytes, endothelium, and smooth muscle cells.[19] Bone marrow hemangioblasts add to the development of new vessels, and mesenchymal stem cells can transdifferentiate into functional cardiomyocytes.[20] Recently, bone marrow–derived cardiomyocytes were demonstrated in hearts of women who received gender-mismatched bone marrow transplantation.[21] Moreover, bone marrow cellular components secrete a range of cytokines, fibroblast growth factor, and vascular endothelial growth factor,[22] which are involved in the natural process of angiogenesis. Endothelial progenitor cells have been implicated in neovascularization associated with postnatal vasculogenesis and are mobilized to peripheral circulation after acute ischemic events.[23]

In the present study, there is preliminary evidence that in humans, bone marrow–derived mononuclear cells are capable of enhancing perfusion, as shown by significant reductions in reversible stress defects on SPECT ($P=0.02$). Bone marrow–derived cells were purposefully injected into areas of hibernating myocardium. In hibernating areas, the underlying physiological state allows for restoration of myocardial function if myocardial perfusion is improved. We hypothesize that angiogenesis is the mechanism that allowed improvement in myocardial function in the patients in our study. Furthermore, we may speculate that an orchestrated sequence of events that includes not only the presence of the transplanted cells but also the action of cytokines and growth factors and intricate cell-to-cell interactions may all contribute to angiogenesis as an end result. Therefore, the resultant localized increase in contractility at cell injection sites, as seen by a significant increase in mechanical function on EMM, likely occurred as a consequence of an underlying improvement in perfusion. However, we cannot exclude the possibility that the injections themselves stimulated new blood vessel growth and enhanced function through the induction of angiogenic and important growth factors.

TABLE 6. Angiographic and EMM Results for the Treatment Group at 4 Months' Follow-Up (n=13)

	Before Treatment	After Treatment	*P*
LV angiogram			
EDV, cc	213.5±81.6	181±51.3	0.1
ESV, cc	174.1±78.7	133.5±54	0.03
EF, %	20±9	29±13	0.0003
EMM			
Unipolar voltage, mV	10.5±3.5	10.3±2.7	0.65
Local linear shortening, %	5.7±3.7	10.8±7.5	0.0005

The homing process, which results in cell engraftment, may also play a key role in the success of cell therapy. After acute events, serum vascular endothelial growth factor levels rise significantly,[23] and it is likely that homing signals may be more intense in acute and subacute ischemic syndromes. In our patients, all of whom had chronic disease, we opted to perform transendocardial cell-therapy delivery because we believe that homing signaling may not be as intense and, therefore, might not be optimal for cell engraftment. It is also likely that a smaller number of cells is required to achieve the desired effect.

EMM technology has been widely confirmed to be accurate for delineating and identifying scarred and viable myocardium and for differentiating degrees of infarct transmurality.[11,12,24] EMM thus offers a theoretical benefit over surgical or intracoronary approaches because viability of the site can be determined before each injection. Injections would then be performed only to targeted, viable areas of hibernating myocardium. Many treated sites targeted in this study were in areas of totally occluded epicardial vascular beds, making intracoronary delivery impossible. Furthermore, potential ischemia provoked by coronary manipulation is avoided. This approved procedure seemed safer for these chronically ill, high-risk patients because it avoided associated surgical morbidity and mortality.

Tse et al[7] recently demonstrated improvement in myocardial perfusion and segmental contractility after ABMMNC transendocardial injections. Those results are somewhat similar to results of the present study, although Tse and colleagues did not see improvement in global EF. The main difference between the studies is the significant baseline LV dysfunction present in our group (mean EF, 20%) as compared with a normal mean EF (56.9%) in the Tse study.[7] The preliminary data of Tse and colleagues also suggest the relative safety of the procedure.

The use of transendocardial delivery proved to be safe in our study, as cellular therapy was successfully delivered in every case without any major periprocedural events (eg, death, myocardial infarction, ventricular arrhythmias, cardiac perforation, pericardial effusion, or development of intramyocardial tumor). Troponin levels increased by a small but significant amount, consistent with delivery via intramuscular injection (Table 4), but the absolute rise was relatively small biologically. The stability between levels of CRP in the treatment and control groups suggests that we did not initiate a significant inflammatory reaction with cell injection.

The major limitations of this study are the small number of patients enrolled and the study design, which limits conclusions about efficacy. Because of ethics committee concerns, the control group was not enrolled concurrently with treated patients, did not receive a placebo injection, and did not undergo invasive follow-up. However, treatment and control groups had similar follow-up up to 2 months. The benefits seen in this study with cell therapy could be attributable to the placebo effect seen in phase 1 trials. Potential biases include selection bias (eg, tertiary hospital population) and investigator bias when assessing symptoms at follow-up (CCSAS and NYHA class) although echocardiographic, angiographic, and SPECT studies were read blindly. In addition, smaller LV volumes and a trend toward higher EFs were present in the control group. However, both groups were matched in terms of demographics, medication use, baseline laboratory values, functional status classification, treadmill workload, and $\dot{V}O_2$max. More importantly, similar baseline reversible and fixed ischemic defects were present in both groups, as one of the most important end points assessed in this study was the amount of reversible perfusion defect at follow-up. The end point of contractility is more difficult to evaluate in light of the differences between the groups at baseline; however, changes in opposite directions occurred at follow-up. In addition, the slightly better LVEFs and smaller hearts should logically have biased results against the cell-treated group.

Although the mechanisms by which cell therapy confers clinical benefit are not well understood, correlation between cell phenotype subpopulation analysis and long-term clinical outcomes is beyond the scope of the present study. Future analyses will be performed in this regard when longer-term follow-up is available.

The treatment of patients with heart failure has become increasingly important given the growing number of cases and their economic impact on the healthcare system.[25,26] More aggressive and widespread therapy in patients with chronic, ischemic heart failure will ultimately lead to a population harboring more advanced disease with a potential yearly mortality rate as high as 50%.[27] For these patients, therapeutic options remain limited. The very high-risk nature of the patient population represented in our study cohort is underscored by the fact that there was a death in both the control and the treatment groups. However, the significant improvement in LVEF noted in the treatment group on angiographic follow-up at 4 months (from 20% to 29%) may imply an improved clinical state and, it is hoped, provide some reduction in risks for the future.[28]

Conclusion

In this initial prospective, nonrandomized, open-label study in no-other-option coronary artery disease patients with LV dysfunction, we noted improvement in symptoms, cardiac function, and perfusion with transendocardial ABMMNC therapy, without any clinical evidence of significant harm from the procedure itself. We believe there may be clinical potential for this relatively novel therapy. Further investigation in a larger, randomized trial is warranted.

Acknowledgments

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